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APPLICATION NO.	FILED DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
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EXAMINER

ART UNIT	PAPER NUMBER
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8

DATE MAILED:

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

please see attached

Office Action Summary

Application No.

09/227,742

Applicant(s)

Bloom et al.

Examiner

Remy Yucel

Group Art Unit

1636



☒ Responsive to communication(s) filed on 12 July 1999 and 24 August 1999

This action is **FINAL**.

Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire three month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) 45-69 is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) 45-69 is/are rejected.

Claim(s) _____ is/are objected to.

Claims _____ are subject to restriction or election requirement.

Application Papers

See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been received.

received in Application No. (Series Code/Serial Number) _____.

received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 5

Interview Summary, PTO-413

Notice of Draftsperson's Patent Drawing Review, PTO-948

Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

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DETAILED ACTION

Claims 45-69 are pending in the application.

This Office action is in response to the amendment filed 12 July 1999.

Oath/Declaration

Receipt of the Oath/Declaration submitted in response to the Office action mailed 12 April 1999 is acknowledged. Addition of the two provisional applications from which Applicant claims benefit is also acknowledged. However, as set forth previously, on the first page of the declaration the statement "the specification is attached hereto *unless* (emphasis added) the following box is checked" appears and the box is not checked. Because this Oath/Declaration is not one that was attached to the parent application, 08/826,426, the box must be checked to so indicate. Correction is appropriate.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

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Claims 45-69 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-10 and 13-16 of U.S. Patent No. 5,891,692 (A).

Although the conflicting claims are not identical, they are not patentably distinct from each other because they are drawn to either increasing transformation ability or viability by increasing unsaturated fatty acid content of bacterial membranes.

A double patenting rejection had been made in the Office action mailed 12 April 1999 which Applicant contends is rendered moot in light of cancellation of claims 1-44. This argument has been considered but is not found persuasive.

Instant claims 45-57 and 60-67 are unpatentable over claims 2-10, 15 and 16 of U.S. Patent No. 5,891,692. Instant claims 58 and 59 are unpatentable over claim 1 of U.S. Patent No. 5,891,692. Instant claims 68 and 69 are unpatentable over claims 13 and 14 of U.S. Patent No. 5,891,692. The subject matter of the patented claims, which are drawn to increasing transformation ability of bacteria and viability of bacteria by increasing unsaturated fatty acids in bacterial membranes through transformation with a gene which directly or indirectly brings about said increase is completely encompassed by the subject matter of the instant claims which are not limited to a particular method of increasing fatty acids in the bacterial membranes. Without a terminal disclaimer, there would be an improper timewise extension of the "right to exclude" granted for the invention of the patented claims by U.S. Patent No. 5,891,692 should a patent issue from the instant application.

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Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless --

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 45-49 and 51-56 are rejected under 35 U.S.C. 102(b) as being anticipated by Inoue *et al.* (U. IDS reference AS11).

The instant claims are drawn to methods of enhancing transformation ability of bacteria comprising increasing the fatty acid content of the membrane of said bacteria.

Inoue *et al.* teach enhanced transformation obtained with bacteria grown at 18 °C (or 25 °C) prior to being processed to make competent cells. After growth at 18 °C (or 25 °C), the cells are either used directly for transformation or put into aliquots and frozen by immersion in liquid nitrogen for later use. Inoue *et al.* teach enhanced or increased transformation efficiencies with bacterial cells (*E. coli*) grown at temperatures below the optimum temperature of 37 °C (see for example page 25, second column). Inoue *et al.*'s method inherently increases the amount of unsaturated fatty acids in the membranes of the bacterial cells.

It was well known in the art that growth of bacterial cells (for example *Escherichia*) below their temperature optimum results in membranes with greater levels of unsaturated fatty acids with respect to bacterial cells grown at higher temperatures (i.e. 37 °C). Ulrich *et al.*

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(reference V, J. Bact., Vol. 154, pages 221-230) and de Mendoza *et al.* (reference W, JBC, Vol. 258, pages 2098-2101), already made of record in the previous Office action clearly teach that fatty acid composition of bacteria change as a function of growth temperature. Both references teach that there is an increasing proportion of unsaturated fatty acids in the bacterial membranes at lower growth temperatures and attribute the observed increase to an increase in *cis*-vaccenic acid synthesis. Both references also teach that the increasing proportion of unsaturated fatty acids results in more fluid membranes. Reference AT5, de Mendoza *et al.* (reference X, TIBS, February 1983) on Applicant's IDS also corroborates the above teachings. Finally, Van Alphen *et al.* (reference Y, Eur. J Biochem., Vol. 101, pages 571-579) teach an increase of unsaturated fatty acids in membranes of *Escherichia* grown at 12 °C and discuss the changes in phase transitions (from solid or liquid or fluid) brought about by increases in unsaturated fatty acids in the membranes. The above papers date from 1979 and 1983, more than 10 years prior to Applicant's (effective) filing date and indicate that with growing bacterial cells below their temperature optimum, there is a concomitant increase in the amount of unsaturated fatty acids found in the membranes. Thus, Inoue *et al.* teach that which is recited by the instant claims.

Claims 58-69 are rejected under 35 U.S.C. 102(b) as being anticipated by de Mendoza *et al.* (X, IDS reference AT5).

The instant claims are drawn to methods enhancing viability of bacteria after cold storage comprising increasing the fatty acid content of the bacterial membranes.

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de Mendoza *et al.* teach that normal cell function requires membranes that are largely fluid and that in response to lower temperatures, the membranes undergo a reversible change of state from fluid, fluid-like state to a hexagonally ordered array of the fatty acid chains (becoming "solid" or more rigid). They teach that bacteria regulate their lipid phase transition in response to temperature to ensure fluid fluid-like membranes for normal cell function and do so by regulating the amount of unsaturated fatty acids in their membranes (order-disorder transition). They teach that by developing a regulatory mechanism based on β -ketoacyl-ACP synthase II, bacteria (for example *Escherichia*) are able to lower the temperature of the order-disorder lipid phase transition to optimize membrane function at lower temperatures (such as those recited in the instant claims). The bacteria do so by increasing the synthesis of *cis*-vaccenic acid and thus increase the amount of said unsaturated fatty acid in their membranes.

They also describe experiments in which the copy number for *fabB* is increased in a *fabF1* strain. In these cells the increase in *cis*-vaccenic acid synthesis is not temperature dependent, but results in increased levels of the unsaturated fatty acid in the bacterial membrane.

By optimizing membrane function to ensure fluid, fluid-like membranes at lower temperatures, enhanced viability is therefore (inherently) achieved because these membranes are less susceptible to "fractionation" which occurs after freeze-thaw cycles (a known method by which bacteria are disrupted) than rigid membranes with higher levels of saturated fatty acids.

Claims 58-63 and 66 are rejected under 35 U.S.C. 102(b) as being anticipated by Emtseva *et al.* (U').

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The instant claims are drawn to methods of enhancing viability by increasing the fatty acids in membranes of bacteria.

Emtseva *et al.* teach increased viability of bacterial cells (including gram-negative *Erwinia*, an enteric bacteria that is closely related to *Escherichia*) when the bacteria are cultivated in the presence of oleic acid which is an unsaturated fatty acid. They report that supplementing the growth medium with oleic acid alters the structure of the cells and that the change results in increase viability. Therefore, Emtseva *et al.* teach that which is recited by the instant claims.

Claims 58-61 and 66-68 are rejected under 35 U.S.C. 102(b) as being anticipated by Tsien *et al.* (V') or Kole *et al.* (W').

The instant claims have been described above.

Tsien *et al.* teach that bacteria, specifically *Streptococcus mutans* constructs its membrane with excess fluidity for increased survival at suboptimal temperatures. They teach that by shifting the growth temperature from 37 to 24 °C, membrane lipids became enriched with unsaturated fatty acids. Bacteria grown at the lower temperature also exhibited higher growth rates when transferred to 10 °C than bacteria transferred from 37 °C. They teach that the increased fluidity in the membranes of cells grown at 24 °C results in the observed increased capacity to grow (viability) at 10 °C. Thus, Tsien *et al.* teach that which recited by the instant claims.

Kole *et al.* teach a screening method to identify strains or mutants of *Leuconostoc oenos* which have altered fatty acid distribution relative to wildtype bacteria. They teach that these

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mutants have increased viability after lyophilization. Kole *et al.* teach that resistance to bovine bile is used as a screen to identify mutants with altered fatty acid distribution. At Table 2 they illustrate that a bovine bile resistant strain contains higher levels of C18:1 fatty (vaccenic) acid than does a bovine bile sensitive strain. Kole *et al.* reiterate the role of lipid packing and membrane fluidity (or lack thereof) in bacterial death due to freeze damage. Kole *et al.* teach that an increase in this fatty acid and survival after freezing was observed for other bacteria and that this may be the mechanism by which bovine bile resistant strains of *Leuconostoc oenos* achieve enhanced viability. Thus, Kole *et al.* teach that which is recited by the instant claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35

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U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Claims 64, 65-67 are rejected under 35 U.S.C. 103(a) as being unpatentable over Emtseva *et al.* (U') in view of either de Mendoza *et al.* (W) or Van Alphen *et al.* (Y)

The instant claims are drawn to enhancing the viability of bacteria of the genus *Escherichia* as well as the species, *E. coli* by increasing fatty acids in the bacterial membranes. Claims 65-67 are drawn to increasing specific fatty acids. The teachings of Emtseva *et al.* were presented above. They demonstrated increased viability of different bacteria, including the enteric, gram-negative bacterium *Erwinia* by supplementing the growth medium with oleic acid. They teach that by exposing the bacteria to increased oleic acid, structural changes occurred that resulted in increased viability of the bacteria.

The teachings of de Mendoza *et al.* (W) and Van Alphen *et al.* (Y) have been discussed above. Applicant's attention is drawn particularly to Table II (page 2100) and Table 1 (page 575), in the respective references. Both tables illustrate that there is an increase in unsaturated fatty acids (C16:1 and C18:1) in bacteria grown at a temperature that is lower than the optimum 37 °C. Both references teach that the increase in unsaturated fatty acids results in changes of membrane structure such that the membranes are in an increased fluid-like phase instead of the ordered arrays which are indicative of the solid phase.

The ordinary artisan would have been motivated to modify the method taught by Emtseva *et al.* to increase the levels of vaccenic acid or palmitoleic acid by supplementing the growth

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medium with these unsaturated fatty acids to increase viability of bacteria (*Escherichia*, specifically *E. coli*). The ordinary artisan would have an expectation of success because Emtseva *et al.* teach that by supplementing the growth medium with an unsaturated fatty acid, the viability of another enteric, gram-negative genus, *Erwinia* was enhanced. The ordinary artisan would have further been motivated to study the increase in viability with the addition of different unsaturated fatty acids to ascertain if the addition of a particular unsaturated fatty acid or a particular combination of unsaturated fatty acid resulted in varying degrees of enhanced viability. Thus, the invention as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made.

Claim Rejections - 35 USC § 112

Claims 45-69 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for increasing the percentage of unsaturated fatty acids in the membrane, does not reasonably provide enablement for increasing the fatty acid content of the membrane. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

It is noted that this issue was raised in the Office action mailed 12 April 1999 as evidenced by the discussion of the term "alteration" (see page 5) and the discussion on page 7, regarding the content of the unsaturated fatty acids. The following factors have been taken into consideration.

The nature of the invention. The nature of the invention is enhancement of

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transformation ability and viability by increasing fatty acids in the membranes of bacterial cells.

The state of the prior art and the predictability or unpredictability of the art. The prior art teaches increased transformation ability and viability with increased fluidity of bacterial membranes which is a result of higher unsaturated fatty acid content (relative to saturated fatty acids) of the bacterial membranes. The art only recognizes that an increase in unsaturated fatty acids result in enhanced transformation ability and viability. Thus, given the teachings of the prior art it is unpredictable that increases in saturated fatty acids results or total fatty acid content (no change in ratio of unsaturated to saturated fatty acids) will result in the same enhanced transformation ability and viability.

The amount of direction or guidance presented in the specification and the presence or absence of working examples. The specification only illustrates enhanced transformation ability and viability in the presence of increased levels of unsaturated fatty acids relative to saturated fatty acids in bacterial membranes. Applicant's disclosure does not demonstrate (or teach) that enhanced transformation ability and viability of bacteria is achieved with an increase in total fatty acid content (no change in the ration of unsaturated to saturated fatty acids) or with an increase in saturated fatty acids in bacterial membranes. Applicant's disclosure is drawn to the increase in the proportion of unsaturated fatty acids in the membrane. It is noted that the parent application which has issued as U.S. patent 5,891,692, is drawn to increasing the proportion of unsaturated fatty acids in bacterial membranes.

The breadth of the claims. The claims are broad because they are drawn to increasing

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fatty acids in bacterial membranes which reads on increasing the total level of fatty acids with no change in the ratio of unsaturated to saturated fatty acids, or increasing the level of saturated fatty acid relative to unsaturated fatty acids or the level of unsaturated fatty acids relative to saturated fatty acids.

The quantity of experimentation. As discussed above, the prior art recognizes enhanced transformation ability and viability in the presence of increased levels of unsaturated fatty acids relative to saturated fatty acids in bacterial membranes. The specification corroborates the teachings of the prior art, but fails to teach the skilled artisan how to achieve enhanced transformation ability and viability by increasing the level of total fatty acids with no change in the ratio of unsaturated to saturated fatty acids or by increasing the level of saturated fatty acids. In the absence of specific teachings and guidance from the specification, the skilled artisan would resort to empirical experimentation to practice the full scope of the claimed invention with no expectation of success. The skilled artisan would not have an expectation of success because of the teachings of the prior art which clearly demonstrate that the fluidity of bacterial membranes is the result of an increase in the level of unsaturated fatty acids in the membranes and that increased fluidity of the membranes leads to enhanced transformation ability and viability. This level of experimentation with no expectation of success would be undue on the part of the skilled artisan. Applicant may obviate this rejection by limiting the claims to an increase in the proportion of unsaturated fatty acids in the membrane and adopting the language of U.S. Patent 5,891,692.

Claims 50 and 62 have an additional enablement issue in that outside of transformation

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with exogenous DNA to increase the proportion of unsaturated fatty acids in bacterial membranes (claims to which have issued in U.S. Patent 5,891,692) Applicant has not taught how an increase in copy number of one or more gene is achieved. For example, Applicant discloses a single alternative method by which mutants with increased levels of unsaturated fatty acids in their membranes are identified and which have enhanced viability and transformation ability. However, the specification fails to disclose the nature (and number) of the mutation(s) which results in the observed increase of the level of unsaturated fatty acids (and therefore the desired phenotypes). Thus, it is not predictable that an increase in copy number of one or more genes has occurred in the disclosed mutant strain. Therefore, Applicant is enabled for methods involving increases in copy number of one or more genes only through transformation with exogenous DNA. Applicant fails to disclose or teach other methods by which an increase in copy number of genes may be achieved. As noted immediately above, this embodiment was already been patented by Applicant.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 45-69 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The methods do not recite any method steps which result in increasing the fatty acid

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content of the membrane of a bacterium, thus the metes and bounds of the claims cannot be established.

Claims 45-69 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

These claims contain the recitation "increasing the fatty acid content of the membrane" however the metes and bounds of the claim cannot be established because it is not clear what Applicant means. Does the recitation refer to an increase in the total amount of (any) fatty acids or an increase in unsaturated fatty acids but not saturated fatty acids or increase in unsaturated fatty acids but not unsaturated fatty acids, or a change in the ratio between the two?

Claim Objections

Claims 52, 53, 64 and 65 are objected to because of the following informalities: while the wording of these claims is not incorrect, may be improved by the insertion of --of the genus-- after "bacteria are" in claims 52 and 64; and --of the species-- after "bacteria are" in claims 53 and 65. This language reflects the language of U.S. Patent 5,891,692 and is appropriate for referring to Latin genus and species designations. Correction is appropriate.

Conclusion

Certain papers related to this application may be submitted to Art Unit 1636 by facsimile transmission. The faxing of such papers must conform with the notices published in the Official Gazette, 1156 OG 61 (November 16, 1993) and 1157 OG 94 (December 28, 1993) (see 37 CFR


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§ 1.6 (d)). The Group 1600 FAX numbers are (703) 308-4242 or (703) 305-3014. Unofficial faxes may be sent to the examiner at (703) 305-7939. NOTE: If applicant *does* submit a paper by fax, the original signed copy should be retained by Applicant or Applicant's representative. NO DUPLICATE COPIES SHOULD BE SUBMITTED so as to avoid the processing of duplicate papers in the Office.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Remy Yucel, Ph. D. whose telephone number is (703) 305-1998. The examiner can normally be reached on Monday through Fridays from 8:30 am to 5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. George Elliott can be reached at (703) 308-4003.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.


REMY YUCEL, Ph.D.
PATENT EXAMINER

Remy Yucel, Ph. D.
September 21, 1999